

We claim:

- 5 1. A method for increasing the content of one or more selected amino acids in a selected tissue or organ of a plant comprising transformation of a plant with at least one recombinant nucleotide sequence construct comprising tissue or organ specific regulatory sequences driving transcription during selected stages of morphogenesis, the construct being operably linked to a chimeric nucleotide sequence comprising:
- 10 (i) a nucleotide sequence encoding a carrier protein comprising a plant specific protein enabling targeted expression of an amino acid enriched protein in the selected tissue or organ of the plant; said nucleotide sequence lacking a termination codon and being fused in frame with
- 15 (ii) at least one nucleotide sequence comprising a selected optimal number of codons encoding an amino acid sequence comprising a selected combination of one or more amino acid residues, wherein said optimal number of codons is selected by determining the number allowing a stable translation;
- 20 said recombinant nucleotide sequence construct enabling stable targeted expression of the selected amino acid enriched carrier protein having a stable polyamino acid extension in the selected tissue or organ of the plant.
- 25 2. The method according to claim 1, wherein the construct with the optimal number of codons allowing stable translation of the polyamino acid extension is selected using a cell free *in vitro* translation (IVT) system.
- 30 3. The method according to claim 1, wherein the targeted expression in the selected tissue or organ of the plant is enabled by a carrier protein having a stable polyamino acid extension and intact biological functions as compared to a corresponding unmodified carrier protein.
4. The method according to claim 1, wherein the construct enabling stable targeted expression of the carrier protein having a polyamino acid extension is selected with a

transient expression assay, comprising a nucleotide sequence encoding a reporter protein fused in frame with the construct according to claim 1.

5 5. The method according to claim 1, wherein the construct enabling stable targeted expression of the carrier protein having a polyamino acid extension is selected by detecting the expression of a reporter protein in a plant cell.

6 The method according to claim 1, wherein the construct enabling a stable targeted expression of the carrier protein having a polyamino acid extension in a selected tissue or
10 organ of a plant is obtainable by a method comprising the steps:

(a) fusing the recombinant nucleotide sequence construct of claim 1 in frame with a nucleotide sequence encoding a reporter protein;

(b) selecting in a cell free translation (IVT) system a construct with an optimal number of codons allowing stable translation of a polyamino acid extension;

15 (c) introducing the construct obtained in step (b) into a plant cell;

(d) selecting with a transient expression assay constructs enabling stable targeted expression of the carrier protein having a polyamino acid extension by detecting the expression of the reporter protein in a plant cell;

20 (e) removing the nucleotide sequence encoding the reporter gene from the constructs selected in step (d) to obtain a construct lacking the nucleotide sequence encoding the reporter protein;

(f) transforming a plant with the construct obtained in step (e).

7. The method according to claim 6, wherein the construct obtained in step (b) and comprising a nucleotide sequence encoding a reporter protein is introduced into a plant cell
25 by microprojectile bombardment.

8. The method according to claim 6, wherein the construct providing stable targeted expression of the reporter protein is selected and subsequent to removal of the nucleotide sequence encoding the reporter protein is introduced into plants using an *Agrobacterium*
30 mediated transformation.

9. The method according to claim 1, wherein the codons encoding the polyamino acid

extension comprises histidine, cysteine, methionine, glycine, lysine, tryptophan, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, serine, threonine, arginine, aspartate, glutamate, asparagines, glutamine encoding codons or any combination thereof.

5 10. The method according to claim 1, wherein the number of codons encoding the selected polyamino acid extension is from four to eighty.

11. The method according to claim 1, wherein the selected tissue or organ of a plant is a seed.

10 12. The method according to claim 1, wherein the selected tissue or organ of the plant is a cell wall or a cell membrane.

13. The method according to claim 1, wherein the selected tissue or organ of the plant is an oil body.

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14. The method according to claim 1, wherein the plant specific carrier protein enabling stable targeted expression in a plant tissue or organ is a protein functioning in the intracellular trafficking pathway of the plant.

20 15. The method according to claim 14, wherein the carrier protein is a cell wall protein or plant viral protein.

16. The method according to claim 15, wherein the carrier protein comprises oleosin, caleosin, steroleosin, cruciferin, napin or a plant viral movement protein.

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17. The method according to claim 16, wherein the carrier protein is a movement protein of Tobacco mosaic virus (TMV MP).

30 18. The method according to claim 1, wherein the regulatory sequence is a promoter expressing during embryogenesis.

19. The method according to claim 1, wherein the regulatory sequence comprises a napin

(NAP), a Cauliflower mosaic virus 35S (35S), a chimeric hybrid (HYB), a 19S, a napalin, a phaseolin, a steroleosin, a caleosin, a cruciferin, an Alfalfa mosaic virus (AMV), a heat-shock, an albumin 2S or an oleosin promoter.

20. The method according to claim 19, wherein the napin (NAP) promoter is a napin (NAP) promoter of *Arabidopsis thaliana*.

21. The method according to claim 19, wherein the hybrid (HYB) promoter is a chimeric promoter comprising an enhancer sequence of the CaMV 35S promoter and the entire napin (NAP) promoter.

22. The method according to claim 1, wherein stable targeted expression of the carrier protein having a polyamino acid extension is provided by using a movement protein expressing under the control of a napin (NAP) promoter.

23. The method according to claim 6, wherein the reporter protein is a nucleotide sequence encoding a detectable protein.

24. The method according to claim 6, wherein the reporter protein is a fluorescent protein.

25. The method according to claim 6, wherein the reporter protein is a green fluorescent protein (GFP), a red fluorescent protein, a β -glucuronidase, an obelin or a luciferase.

26. A recombinant nucleotide sequence construct for increasing the content of one or more selected amino acids in a selected tissue or organ of a plant, wherein the construct which enables stable targeted expression of an amino acid-enriched protein having a polyamino acid extension in a selected tissue or organ of a plant comprises a tissue or organ specific regulatory sequence driving transcription during selected stages of morphogenesis operably linked to a chimeric nucleotide sequence comprising;

(a) a nucleotide sequence encoding a carrier protein comprising a plant specific protein enabling stable targeted expression in a selected tissue or organ of a plant; said nucleotide sequence lacking a termination codon and being fused in frame with

(ii) at least one nucleotide sequence comprising a selected optimal number of codons allowing stable translation of the polyamino acid extension, and encoding an amino acid sequence comprising a selected combination of one or more amino acid residues.

- 5 27. The construct according to claim 26, wherein the construct, which enables stable targeted expression in the selected tissue or organ of the plant comprises a nucleotide sequence encoding a carrier protein having a polyamino acid extension and intact biological functions as compared to the corresponding unmodified carrier protein.
- 10 28. The construct according to claim 26, wherein the construct enabling the selection of a construct allowing targeted expression of the carrier protein having a polyamino acid extension comprises a nucleotide sequence encoding a reporter protein fused in frame with the chimeric nucleotide sequence according to claim 26.
- 15 29. The construct according to claim 26, wherein the plant specific carrier protein enabling stable targeted accumulation in a plant tissue or organ comprises a protein functioning in the secretory intracellular trafficking pathway.
- 20 30. The construct according to claim 26, wherein the carrier protein is a plant cell wall protein or plant viral protein.
31. The construct according to claim 26, wherein the carrier protein is oleosin, caleosin, steroleosin, cruciferin, napin or a plant viral movement protein.
- 25 32. The construct according to claim 26, wherein the carrier protein is a movement protein of Tobacco mosaic virus (TMV MP).
- 30 33. The construct according to claim 26, wherein the codons comprise histidine, cysteine, methionine, glycine, lysine, tryptophan, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, serine, threonine, arginine, aspartate, glutamate, asparagines, glutamine encoding codons or any combination thereof.

34. The construct according to claim 26, wherein the optimal number of codons is from 4 to eighty.

35. The construct according to claim 26, wherein the regulatory sequence is a promoter
5 expressing during embryogenesis.

36. The construct according to claim 26, wherein the regulatory sequence comprises a napin
(NAP), a Cauliflower mosaic virus (CMV 35S), a chimeric hybrid (HYB), a19S, a nopaline, a
phaseolin, a steroleosin, a caleosin, a cruciferin, an Alfalfa mosaic virus (AMV), a heat-
10 shock, an albumin 2S or an oleosin promoter.

37. The construct according to claim 26, wherein the promoter is a napin (NAP) promoter of
Arabidopsis thaliana.

38. The construct according to claim 36, wherein the chimeric hybrid (HYB) promoter
comprises an enhancer sequence of the CaMV 35S promoter and the entire napin (NAP)
promoter.

39. The construct according to claim 28, wherein the reporter protein is a detectable protein.

40. The construct according to claim 28, wherein the reporter protein is a fluorescent protein.

41. The construct according to claim 28, wherein the reporter protein is green fluorescent
protein (GFP), red fluorescent protein, β -glucuronidase, obelin or luciferase.

42. The construct according to claim 28, wherein the construct comprises a nucleic acid
sequence encoding a carrier protein having a histidine enriched extension and a green
fluorescent protein (GFP).

43. The method according to claim 1 for producing a composition comprising in plant
material an amino acid-enriched carrier protein having a polyamino acid extension, wherein
the content of amino acids in the plant material obtained by the method as compared to the

amino acid content in a corresponding unmodified wild type plant is at least 2:1.

44. The method according to claim 43 for producing an amino acid-enriched feed of an oil cake obtained after recovery of oil from plants.

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45. The construct according to claim 26 for producing a composition comprising in plant material an amino acid-enriched carrier protein having a polyamino acid extension, wherein the content of amino acids in the plant material obtained by the method as compared to the amino acid content in a corresponding unmodified wild type plant is at least 2:1.

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46. A plant transformed with the construct according to claim 26.

47. A plant transformed with the construct according to claim 28.

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48. A plant cell transformed with the construct according to claim 26.

49. A plant cell transformed with the construct according to claim 28.

50. A plant cell-line transformed with the construct according to claim 26.

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51. A plant cell-line transformed with the construct according to claim 28.

52. A composition obtainable by the method according to claim 1, which composition comprises in plant material an amino acid-enriched carrier protein having a polyamino extension, in which composition the content of amino acids in the plant material obtained by the method as compared to the amino acid content in a corresponding unmodified wild type plant is at least 2:1.

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